Hemodynamics in Nailfold Capillaries of Patients with Systemic Scleroderma: Synchronous Measurements of Capillary Blood Pressure and Red Blood Cell Velocity

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There is increasing evidence that endothelial damage occurs at a very early stage during the course of systemic scleroderma. Endothelial damage is accompanied by impaired microvascular function, which has clearly failed in patients with systemic scleroderma, as evidenced by necrosis of the fingertips in severe cases. We investigated two important determinants of microvascular function, namely capillary blood pressure and capillary red blood cell velocity, simultaneously in the same capillary. In patients with systemic scleroderma and in healthy volunteers matched for age and sex, capillary blood pressure was measured by direct cannulation and capillary red blood cell velocity by video microscopy. Capillary blood pressure and capillary red blood cell velocity were significantly lower in patients (14.27 ± 4.34 mmHg, 230 ± 310 μm per s) than in healthy controls (19.06 ± 3.69 mmHg, $p < 0.008$, and 910 ± 240 μm per s, $p < 0.003$) at an ambient temperature of 22°C, whereas no significant difference in skin temperature was observed (23.7 ± 0.9°C *vs* 24.7 ± 1.9°C) and no occlusion of finger arteries was detected. Capillary blood pressure in enlarged capillaries did not differ from that in normal-shaped capillaries in the patients (correlation of diameter and capillary blood pressure, $R^2 = 0.04$), which was also the case with capillary red blood cell velocity ($R^2 = 0.13$). Capillary pulse pressure amplitude and capillary red blood cell velocity showed a strong correlation ($R^2 = 0.81$), suggesting that the pressure gradient across the capillary loop, which is the driving force for capillary red blood cell velocity, was mainly dependent on precapillary resistance. These observations reflect the inadequate microvascular function in systemic scleroderma, which may be due mainly to a pathophysiologic functional increase in precapillary resistance, even at comfortable ambient temperatures. ***Key words: capillary hemodynamics/microcirculation/Raynaud's phenomenon/skin blood flow. J Invest Dermatol 110:982–985, 1998***

The etiology of systemic scleroderma (SSc) is still under investigation. Previous studies have shown that pathophysiologic changes in the vascular endothelium seem to be followed by increased collagen production in the pericapillary space. Studies of capillary morphology revealed a typical "scleroderma-like" pattern in patients with SSc (Maricq *et al*, 1980). These changes may precede the detection of characteristic antibodies by years. The increased serum levels of von Willebrand's factor and thrombomodulin indicate endothelial cell damage (Kahaleh *et al*, 1981; Mercie *et al*, 1995). In addition, an increase in capillary permeability was detected by fluorescence videomicroscopy, indicating a functional impairment of transcapillary filtration (Bollinger *et al*, 1986). This is accompanied by histopathologic changes with thinning of the basement membrane, endothelial swelling, and proliferation (Thompson *et al*, 1984).

The mechanisms behind this endothelial damage are still under investigation. There is evidence that membrane cofactor protein and decay accelerating factor, which normally protect endothelial cells from damage by autologous C, are downregulated in patients with SSc (Venneker *et al*, 1994). The damaged endothelium may lead to an increased migration of leukocytes and mononuclear cells into the interstitial space, stimulating fibroblast function. Increased expression of endothelial adhesion molecules is associated with microvascular damage in SSc. Molecules such as intracellular adhesion molecule-1, vascular cell adhesion molecule-1, or E-selectin even seem to be correlated with clinical progression or remission (Denton *et al*, 1995). Furthermore, platelets may be activated by the damaged endothelium, stimulating fibroblasts via mediators like platelet-derived growth factor. Co-culture studies have suggested a link between endothelial and fibroblast dysfunction (Denton *et al*, 1996). This may explain the initially perivascular fibrosis (Kahaleh, 1990). These observations suggest that microvascular disturbances may precede fibrosis and may therefore be one of the earliest detectable symptoms of SSc.

Capillary blood pressure (CP) is an important determinant of microvascular function. Fluid filtration and flow rate are governed by the pressure and its gradient across the capillary loop. Microvascular function has clearly failed in SSc, where one of the first clinical symptoms is acral vasospasms, so-called Raynaud's phenomenon, and where severe cases can display stenosis of the arteries in the hands and fingers and morphologic changes in the capillaries accompanied by tissue necrosis of the fingers. In addition, dynamic microvascular studies have revealed significantly lower red blood cell velocities in nailfold capillaries during local cooling without detectable stenosis of the supplying arteries (Meier *et al*, 1978). Despite the importance of CP, however, nothing is known about blood pressure at the capillary level in these patients. In this paper we present our first synchronous

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Abbrevations: CBV, capillary red blood cell velocity; CP, capillary blood pressure; CPPA, capillary pulse pressure amplitude; SSc, systemic scleroderma.

measurements of CP and capillary red blood cell velocity (CBV) in the same capillaries of patients suffering from SSc and compare them with measurements made in age- and sex-matched healthy controls.

MATERIALS AND METHODS

Subjects We examined 11 patients (mean age 49.2 y, range 29–73 y, eight women, three men) during the winter season. The patients suffered from SSc for 8 ± 6.5 y (range 1–22 y). The diagnosis was based on patient history with Raynaud's phenomenon, clinical examination, serologic markers (one patient with anti-topoisomerase-1, five patients with anti-centromere antibodies, and five patients with anti-nucleoli antibodies), and nailfold capillaroscopy (all presented a scleroderma-like pattern; Maricq *et al*, 1982). Nine patients fulfilled the criteria published by LeRoy *et al* for limited scleroderma and two fulfilled the criteria for diffuse scleroderma (LeRoy *et al*, 1988). The average number of Raynaud's attacks in the week before was 1.7 ± 1.1 per d (range 1–4 attacks).

The control group was matched for age and sex (mean age 46.3 y, range 24–72 y, eight women, three men). Three women in each group were postmenopausal and all of them were aged 50 y or older. One of the patients and one of the healthy control were taking the contraceptive pill, none were taking vasoactive drugs. All subjects were asked to abstain from caffeine containing beverages on the day of examination and none were smokers. All patients received duplex sonography of the supplying arteries, including the finger arteries of the examined finger (Trager *et al*, 1993).[1] The study was approved by the local ethics committee and all subjects gave informed consent.

Experimental protocol All experiments were performed in a temperature-controlled environment (22 ± 0.2°C) after a period of half an hour of acclimatization. The subjects lay in a supine position, hand at heart level (mid-axillary line). CP was measured in the nailfold capillaries of the third or fourth finger of the left hand. An electrocardiogram (Hewlett Packard, 78354C, Böblingen, Germany) and finger skin temperature (Mammendorfer Institut für Physik und Medizin, Temperaturmeβmodul, Hattenhofen, Germany) were recorded simultaneously. Blood pressure (Critikon, Dinamap, Norderstedt, Germany) was monitored at the right arm throughout the study. The set-up has been described in detail previously (Hahn *et al*, 1996a).

Visualization of capillaries and cannulation Capillaries are visualized by video microscopy, which makes it possible to record the procedure. A video camera (BC-5, AVT-Horn, Aachen, Germany) was attached to an incident light microscope (lens: L25/0.22 PUT) with a built-in zoom (Leica, Wetzlar, Germany), and lighting was provided by a mercury vapor lamp. The microscopical image was transmitted via the video recorder (U-matic, Sony, Tokyo, Japan) to a black and white monitor (Ikegami Tsushinki, Tokyo, Japan). This system gave an on-screen magnification of between 1 pixel per 1.16 μm and 1 pixel per 0.46 μm.

The capillaries nailfold were cannulated at the top of the capillary loop under a microscope, using micropipettes (Hilgenberger GmbH, Malsfeld, Germany) held in a micromanipulator (Leitz, Wetzlar, Germany). Readings were accepted if the signal was insensitive to an increase in gain, and if capillary blood flow and the shape of the capillary remained unaltered by the procedure. CP was measured in at least three different capillaries.

Pressure measurement To record pressure, we used a servo nulling pressure system (Model 5 A, IPM, San Diego, CA) (Intaglietta, 1973), the successor of the pioneering one used by Mahler *et al* for the first time in humans (Mahler *et al*, 1979). The pressure transducer (P23XL, Statham, CA), amplifier (Mammendorfer Institut, München, Germany), and computer were calibrated against a digital manometer (Digitron Instruments, Hertford, Hertfordshire, U.K.). The signal of the pressure transducer was amplified and low-pass filtered (frequency 20Hz, Mammendorfer Institut, München, Germany). Data digitalization with a digital analog converter (DT 2826, Data-Translation, Marlboro, MA) was followed by computerized data acquisition (DAGO-PC, Gesellschaft für Strukturanalyze, Aachen, Germany). The data were stored on a hard disk for later off-line analysis.

Synchronization of CBV and CP The microscopic image was recorded with a U-matic video recorder (VO 9600P, Sony, Tokyo, Japan) throughout the study. Every single frame of the recorded picture was numbered by a frame code generator (FCG-700, Sony, Tokyo, Japan). By starting the software for data acquisition and the frame code generator simultaneously by a common external trigger (AVT-Horn, Aachen, Germany), we were able to synchronize both signals. In addition, a time code generator (WJ-810, Panasonic, Tokyo, Japan) with a stop watch printed the elapsed time on every half frame, and the elapsed time from the start of measurement is given for the digitized CP data. This allowed us to verify the synchronization of the recording.

Data analysis Computer-based recordings enabled us to use computerized data analysis. The data sampling frequency was 100 Hz for CP and ECG. In addition, the skin temperature of the examined finger and systemic blood pressure were continuously recorded. To evaluate the data, we developed our own software that allowed us to automatically analyze the mean CP and the average pulse wave form by superimposing consecutive pulse waves using the R-wave of the ECG as a timer to indicate the electrical start of the systolic beat (Hahn *et al*, 1996b).

CBV was analyzed later, off line, using a video image analysis system that allows continuous analysis in real time (Pries, 1988; Pries *et al*, 1989). All CBV measurements were performed in the arterial limb of the capillary with the line of measurement ending at the summit of the capillary loop.

The capillary diameter was measured as the visible diameter of the red blood cell column as provided by conventional capillaroscopy in all patients where we were able to measure CBV at the same time. The true diameter is known to be significantly larger, but can only be visualized by staining the plasma, e.g., with indocyanine green (Bollinger *et al*, 1991). The diameter was determined for the arterial limb and the venous limb at a distance of about 30 μm below the apex and in the apex itself.

Statistical methods Data are presented as medians (25% quantile and 75% quantile) or, if appropriate, in means ± SD. Statistical analysis was performed using the Wilcoxon sign rank test and linear regression (Jump, SAS Instituts, Cary, NC).

RESULTS

Good intercapillary and intraindividual reproducibility of CP and CBV, not influenced by capillary diameter Measurements of CP in 18 different capillaries in four healthy controls showed an overall variability of 2.5 ± 0.7%, and measurements in 30 capillaries in five patients varied by 4.1 ± 3.2%. The relative variability in the simultaneously measured CBV was less pronounced in these four healthy controls (14.6% ± 2.4%) than in the corresponding five patients (24.4% ± 22.4%).

In six readings obtained from three healthy controls and from three patients over at least a 45 s period, the maximum and minimum mean CP value measured in 5 s time periods varied by 0–3.1 mmHg, average 0.6 ± 0.34 mmHg. In three patients with a marked variability in measured capillary diameter (arterial limb, 9.7–63.9 μm; apex, 18.7–59.7 μm; venous limb, 9.7–50.8 μm), the overall variability of CP was calculated to be only 5.5% ± 3.6%. In these patients, no linear correlation was found between the capillary diameters and CP based on at least nine readings (arterial diameter, $R^2 = 0.04$; apex diameter, $R^2 = 0.02$; venous diameter, $R^2 = 0.01$).

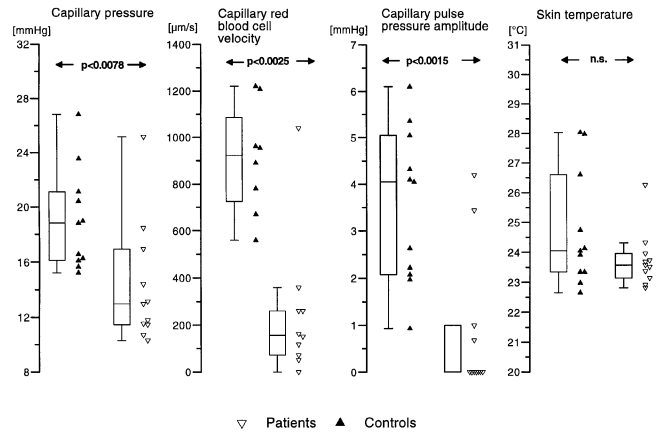

Figure 1. Comparison of mean values of capillary pressure, capillary red blood cell velocity, capillary pulse pressure amplitude, and skin temperature between healthy controls and patients. Median, 25% resp 75% quantile, inner fences and individual values of each subject are given.

[1]Hahn M, Steins A, Jünger M. Farbduplexsonographie der Fingerarterien - Beispielhaft gezeigt an Gesunden und an Patienten mit sekundärem Raynaud Phänomen. *VASA* 45 (suppl.):117, 1995 (abstr.)

Lower CP, capillary pulse pressure amplitude (CPPA), and CBV in patients, even without detectable occlusion of finger arteries and values within the normal range during the late hyperemic phase Mean capillary blood pressure was significantly lower in patients with SSc than in healthy volunteers (n = 11, **Fig 1**), whereas no significant difference was observed in skin temperature and systemic blood pressure (**Table I**). Three of the 11 patients showed values within the normal range (18.5 mmHg, 17.0 mmHg, 25.2 mmHg). All three suffered a Raynaud's attack when they arrived at the hospital with subsequent post-ischemic hyperemia. After half an hour of acclimatization, they still reported unusually warm hands. CBV was increased in these patients as well (1040 μm per s, 680 μm per s, >800 μm per s). In one patient we started measurement right after he entered the laboratory. CP and CPPA dropped during the acclimatization period from 34.2 mmHg to 17.0 mmHg and from 3.1 mmHg to 0.7 mmHg, respectively. CBV dropped as well from 870 μm per s to 117 μm per s.

In addition, CPPA was significantly lower in patients than in healthy controls (**Fig 1**). Only in four patients was a pulse wave detectable. Compared with the age- and sex-matched healthy controls, the pressure amplitude was lower, systolic arrival time of the pulse wave was delayed, and time to peak was increased (**Table I**). Due to the limited number of observations, this was not tested for significance.

Measurements of CP in human subjects are among the most demanding studies in physiology. We also tried to measure CBV synchronously in the same capillary and succeeded in 10 patients and in eight healthy volunteers (**Fig 1**). Simultaneous recordings of CP and CBV were possible for up to 449 s of uninterrupted continuous measurement. **Figure 3** gives the original recording of CBV and CP in a single capillary of a 72 y old female patient with pulsatile pressure and flow. Pulsatile flow was observed in patients even when no CPPA was detectable. The median CBV in patients and that of the age- and sex-matched healthy volunteers are given in **Table I**. Mean CBV correlated well with CPPA (R^2 = 0.81, **Fig 2**) and was significantly lower in patients than in healthy controls (**Fig 1**).

In eight patients no stenosis of the supplying arteries was detectable, in two patients one finger artery showed a single stenosis and in one patient two finger arteries of the examined finger showed stenosis. Mean capillary pressure of the three patients with stenosis of finger arteries remained in the range observed in the patient group as a whole (18.5 mmHg, 14.4 mmHg, 13.0 mmHg).

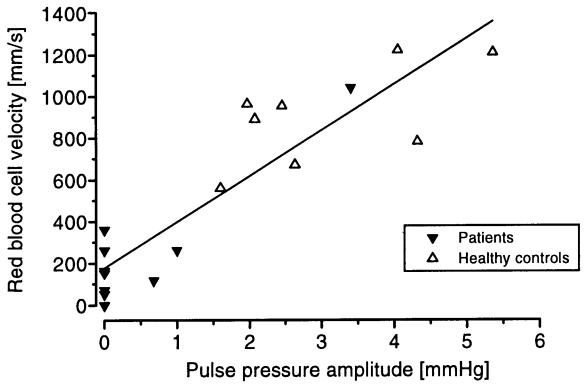

Figure 2. Correlation of CPPA and capillary red blood cell velocity (R^2 = 0.81) measured in the same capillary synchronously.

DISCUSSION

This study reveals a significantly lower CP and CBV in patients with SSc than in age- and sex-matched healthy controls. This marked difference cannot be explained by the small, statistically insignificant difference in skin temperature. This would account for only about 0.5 mmHg based on the study of Shore *et al* (1993). Occlusion of the supplying arteries was not found and the stenosis of finger arteries observed in three patients was not correlated with any difference in mean CP, suggesting that the blood supply to the finger can easily compensate for the reduced flow in one or two of the four finger arteries. In three patients in the late hyperemic phase of a Raynaud's attack, CP values were within the range of healthy volunteers. This would support the idea of impaired microvascular function caused by a pathophysiologic increase of precapillary resistance in these patients, rather than by morphologic stenosis of the supplying arteries.

Two different interpretations should be considered. First, the lower CP in the patients might be the expression of a *per se* unaffected feedback system of pre- to postcapillary resistance. It could be speculated

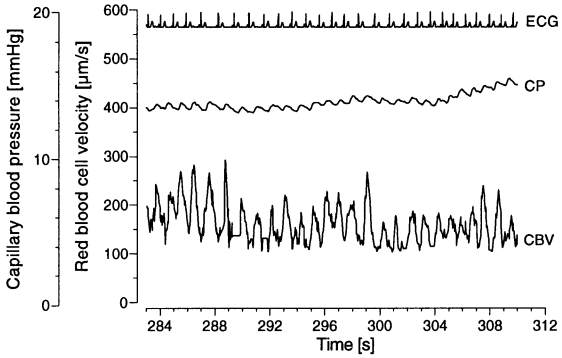

Figure 3. Original recording of a synchronous measurement of CP and CBV in the same capillary with corresponding ECG. Measurement was performed in a hair-pin-shaped nailfold capillary of the first row at heart level. Diameters of arterial and venous capillaries, 22.3 μm and 49.1 μm, respectively; finger temperature, 23.73°C; systolic blood pressure, 145 mmHg; diastolic blood pressure, 70 mmHg.

Table I. Comparison of median values between patients and age and sex matched controls

	Healthy volunteers (n)[a]	Patients (n)	p value
Age [y]	51 [33/56] (11)	50 [37/55] (11)	0.81
Skin temperature (°C)	24.0 [23.3/26.6] (11)	23.6 [23.1/24.0] (11)	0.28
Systolic BP[b] (mmHg)	130 [120/135] (11)	120 [110/145] (11)	0.46
Diastolic BP[b] (mmHg)	75 [70/80] (11)	72 [70/80] (11)	0.59
Mean BP[b] (mmHg)	93 [87/97] (11)	90 [82/95] (11)	0.41
Capillary pressure (mmHg)	18.8 [16.1/21.1] (11)	13.0 [11.5/17.0] (11)	0.0078
CPPA (mmHg)	4.0 [2.1/5.1] (11)	0.0 [0.0/1.0] (11)	0.0015
Systolic arrival time (s)	207 [169/234] (4)	264 [205/292] (4)	–
Mean rise in CPPA (mmHg per ms)	0.017 [0.015/0.025] (4)	0.008 [0.002/0.018] (4)	–
CBV (μm per s)	920 [700/1150] (8)	140 [45/290] (10)	0.0025
Arterial CD[c] (μm)	14.5 [11.4/16.1] (8)	25.6 [21.6/40.2] (10)	0.0014
Summit CD[c] (μm)	18.5 [17.2/21.6] (8)	29.1 [22.9/42.2] (10)	0.0025
Venous CD[c] (μm)	13.8 [11.9/15.3] (8)	28.8 [21.9/39.6] (10)	0.0007

[a]Values are medians (25% quantile/75% quantile); no. of volunteers is given in parentheses for each measurement.
[b]BP, blood pressure.
[c]CD, capillary diameter, measured ≈30 μm below the lower part of the summit of the capillary loop (venous and arterial) and at the middle of the summit of the loop.

that the upregulated precapillary resistance may be caused by increased transcapillary filtration, as observed by fluorescence videomicroscopy, to prevent edema formation (Bollinger *et al*, 1983). But the more likely explanation is an imbalance in the feedback system itself. An imbalance in the vasoconstrictive and vasodilatative mechanisms mainly caused by endothelium cell damage could lead to the observed increase in precapillary resistance even at ambient temperatures (Kahaleh *et al*, 1979). The vasodilative effect of bradykinin, an endothelium-dependent vasodilatator, was weakened, whereas the effect of nitroprusside, an endothelium-independent vasodilator, was no different in patients with Raynaud's phenomenon than in healthy controls (Bedarida *et al*, 1993). We suggest an impaired NO release from the endothelium. On the other hand, increased levels of vasoconstrictive agents like plasma endothelin and thrombomodulin were found in SSc (Zamora *et al*, 1990; Kadono *et al*, 1995). Sclerosis of the surrounding tissue may also change the elasticity of the vessels and reduce their diameter, which would increase precapillary resistance. In addition, structural changes of supplying arteries, mostly finger arteries, were described in patients with SSc and may also contribute to an increased precapillary resistance (Wagner and Alexander, 1993). We could only detect stenosis of finger arteries in three of 11 patients. The values of CP in these patients were within the range observed in the total patient group. In contrast, in three patients during the late hyperemic phase after a Raynaud's attack, CP values were within the normal range. Furthermore, in patients with primary Raynaud's phenomenon, without known structural changes, a significantly lower mean CP and CPPA is known.[2] Therefore we believe that the functional increase in precapillary resistance was the hemodynamically most relevant pathophysiologic factor for decreased finger skin perfusion observed in these patients. In the late course of the disease, structural changes and occlusions of digital arteries may in addition contribute to microcirculatory malfunction. This function has clearly failed, as shown by necrosis of the fingertips in the severe disease stage.

The method described here enabled us to simultaneously measure CP and CBV in human skin capillaries. The movement of fluid across the capillary wall is governed by several factors as described by Starling's equation, of which CP is probably one of the most variable and the oxygen supply is partly controlled by CBV. Slight pulsatile pressure variations were accompanied by a pronounced pulsatility of CBV. Even when no pulsatile pressure was detectable, CBV still showed pulsatile variations. These results are supported by previously described minimal variations in CP accompanied by marked variations in CBV in healthy volunteers (Hahn *et al*, 1996a) and by *in vitro* experiments showing no evidence of yield shear stress using glass micropipettes (Gaethgens, 1987). The observed differences in the variability of CBV and CP in adjacent capillaries further support the idea that very small pressure differences are associated with marked changes in CBV. Shore *et al* found highly reproducible CP values across the nailfold in five individuals (5.4 $\pm$ 2.0%) and very low day-to-day variability in six individuals, with a mean coefficient of variation of 5.2 $\pm$ 3.6% (Shore *et al*, 1995). CPPA and CBV showed a strong correlation. CBV is driven by the pressure gradient across the capillary loop and CPPA depends mainly on precapillary resistance. In other words, the strong correlation suggests that the pressure gradient, and therefore CBV, is mainly dependent on precapillary resistance.

In this study, not only healthy volunteers but also patients showed low variability in CP across the nailfold. The differences observed in CP between capillaries of different diameters were within the range observed in healthy controls. There was no correlation between capillary diameter and CP. This may be due to the fact that adjacent capillaries are closely linked together in a microvascular network of communicating tubes, where only small pressure differences can be realized.

In patients with Raynaud's phenomenon due to SSc, CP and CBV values are decreased even at comfortable ambient temperatures. This seems to be caused primarily by a functional increase in precapillary resistance and leads to a malfunction of skin microcirculation as evidenced by tissue necrosis in severe cases. Further studies will be needed to investigate endothelial function in these patients by determining the effect and mode of action of vasodilatative drugs on skin perfusion, especially during the very early stages of the disease.

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[2]Hahn M, Shore AC, Sandeman DD, Tooke JE. Dynamic measurement of human capillary blood pressure in patients with Raynaud's phenomenon and in healthy volunteers. *Int J Microcirc Clin Exp* 11 (suppl.1):S52, 1992 (abstr.)

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